The Development of Pregnancy Tests

The most recently developed test, using the *Rana pippens* male frog, makes it possible to determine the presence of pregnancy within two to five hours after testing.

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Man's natural curiosity concerning proof of early pregnancy probably extends to the beginning of time; evidence of this interest can be found in Egyptian medical papyri dating back nearly 4,000 years. The *Berlin Medical Papyrus* (1350 B.C.), for instance, describes one such test:

Another test for a woman who will bear or a woman that will not bear. Wheat and spelt; let the woman water them daily with her urine like dates and like Sh'at seeds in two bags. If they both grow she will bear; if the wheat grows it will be a boy; if the spelt grows it will be a girl. If neither grows, she will not bear.

Many of the ancient tests were absurd and bizarre in the light of modern scientific knowledge, but the idea that there was something in the urine of a pregnant woman which would stimulate plant growth or seed germination has persisted in varying forms down through the years.

It was not, however, until the twentieth century that any real progress in scientific research in this field took place. Robbins reports that it was the discoveries of Evans and his associates, Smith and Engle, and Aschheim and Zondek that "made possible the present era of accurate pregnancy diagnosis." These men demonstrated the "complex interrelationship between the ovary and the pituitary body and the urinary reaction of these hormones..." It was discovered that the urine of pregnant women contained large quantities of hormones that were not found in the urine of non-pregnant women. It was also found that these hormones (a complex of anterior-posterior pituitary hormone which has since been broken down into its several parts, one of which is the chorionic gonadotropin mentioned hereafter) would stimulate the ovaries of female animals to ovulate.

Once the basic truths were discovered, each step followed the next until present-day pregnancy tests employing rabbits and frogs came into use. The male frog test, to be discussed in detail later, is the most recent of the reliable procedures developed for the biological detection of early pregnancy.

Evolution of Pregnancy Tests

In 1928 Aschheim and Zondek published the report of their test for pregnancy, using the urine of pregnant women, and immature mice for test animals. This was the first scientific, biological method for the diagnosis of early pregnancy that was nearly 100 per cent accurate. Its accuracy is unsurpassed today, although there are many other reliable tests. The main drawbacks to this test are the longer period of time that is required, and the number of animals that must be used.

Using the basic facts that the injection of gonadotropic hormones into female mammals would cause hyperemia, follicle stimulation, and production of corpus luteum, later workers developed many variants of the test in the attempt to cut down the reaction time.

Friedman developed his modification in 1930 using mature, isolated female rabbits. This test cut the reaction time to forty-eight hours. It is used extensively today, but is comparatively expensive. There is also some possibility for variation in reading the test, based on subjective interpretation.

The next advance in the development of pregnancy testing occurred when it was discovered that mammalian hormones would stimulate ovulation in amphibian animals.

Hogben, working in South Africa in 1930, demonstrated that the female * Xenopus laevis* toad could be stimulated to ovulate spontaneously by the injection of extracts of the anterior lobe of the pituitary gland. He then tried injecting the urine from pregnant women into the toads, and found that the same reaction occurred. This toad does not extrude ova unless it is stimulated by the male toad or, if isolated in the laboratory, only when it is injected with the chorionic gonadotropins. Ova were deposited within twenty-four hours after the injection of the urine when chorionic gonadotropin was present. Although this test cut the reaction time for pregnancy testing to twenty-four hours, it did not prove suitable for widespread use, because the * Xenopus laevis*, a South African clawed toad, was not available elsewhere.

Progressive work with various female toads was continued in an effort to simplify the test and cut down reaction time, but until Mainini published his work in 1947, the male gonads had been ignored.

Mainini used the male *Bufo arenarum* Hensel, a South American toad, with excellent results. He found that the injection of untreated pregnancy urine into this toad caused spermatogenesis in from one to twenty-four hours after injection. All of his tests were run with parallel times using the Friedman modification, and carried out by another laboratory. He reported an accuracy of 96 per cent. This test was inexpensive, easy to carry out, and rapid; furthermore, the end point was clear. The presence of sperm in the toad urine indicated a positive test. He reported no false negatives. The test was also specific. The male toad does not emit sperm in captivity, unless it is stimulated by pregnancy hormones.

Tests trying the effects of other biological preparations such as estrogen, insulin, and thyroxin were all negative. Only extracts of chorionic gonadotropin produced positive results. As the concentrations of the chorionic gonadotropin increased, the positivity and speed of reaction increased. Mainini also ran a series of controls using urine from non-pregnant women and young normal girls. These seventy-seven tests were negative. Thus he proved the specificity of his test.

After a report of Mainini's test was published, American workers began trying it. Because the *Bufo arenarum* Hensel toad was not available, they sought a suitable amphibian native to North America. Almost simultaneously, in 1948, Robbins and Parker (5), and Wiltberger and Miller (6) published papers reporting their work using the male *Rana pippens*, a common North American frog. Their ex-
periments proved the reliability of *Rana pipiens* as a test animal; they found no false positives; the frog test compared favorably with the Friedman test in accuracy; it was rapid; and the animals were plentiful and inexpensive.

Since this test using *Rana pipiens* was first described, many workers have used it, constantly striving to improve it, studying its accuracy, and determining the possible variations caused by different animal temperatures, moisture content, and age of the frogs. Nearly all agree that it is an excellent test for determining early pregnancy (7).

### The Rana Pipiens Male Frog Test

Some knowledge of the anatomy of the frog is necessary if one is to understand the physiology of this test.

The frog testicle is an elongated structure attached to the dorsal side of the pericardial cavity. The organs are attached to the kidney of the same side by a ligament through which pass the collecting tubes which carry spermatozoa. These tubes empty into kidney glomeruli. The kidney glomerulus and the bladder are the common excretory channels for both urine and spermatozoa. This arrangement allows for external fertilization, which is natural with the frog. The gonadotropin hormones contained in the urine of pregnant women, when injected into a frog, are capable of causing a detachment and excretion of spermatozoa. Spermatogenesis is also probably stimulated by these urinary gonadotropins, but it is the former action which explains the rapidity with which spermatozoa are found in the frog's urine following injection with pregnancy urine (8).

The procedure for using the *Rana pipiens* male frog is readily adapted to the office routine of a private physician. Physicians, particularly obstetricians, who will have frequent occasion to perform the test will probably want to keep a supply of frogs on hand. They may be kept in the vegetable drawer of the office refrigerator (suitably labeled) at a temperature of about 40°F. The moss in which they are packed for shipping is placed on the bottom of the drawer, and one-half inch of water is added. The frogs must not be allowed to become dry, but if they are in too deep water, they will drown. The water must be changed at least twice a week to prevent disease. They are not fed. These frogs should be kept under proper refrigeration for many weeks; it is recommended, however, that they not be used after three months of such storage to avoid the use of debilitated frogs. If they are kept in the laboratory in this manner, they are always available for instant use.

Prior to running a test, the frog should be removed from the refrigerator, and placed in a clean, wide-mouth jar with a perforated lid. This allows the animal to reach room temperature before the injection. He reacts more quickly to the hormone when not extremely cold. Frogs kept under refrigeration before use are also found to be more sensitive to the hormone than those kept at room temperature (9).

When a patient has symptoms of pregnancy which are complicated by other conditions, and the need for a pregnancy test is evident, she is instructed to bring about two ounces of early morning urine in a clean bottle. The urine needs no special treatment prior to injection within a reasonable length of time (six to eight hours); but if it is to be kept several days before the test is to be run, the specimen should be refrigerated to prevent deterioration of the labile hormone (10).

To inject the frog, 2½-cc. of urine is placed in a hypodermic syringe, to which is attached a 26-gauge, 1/4-inch needle. Then the worker grasps the frog firmly in one hand so that its legs are controlled, exposing the dorsal lymph sac. With the syringe in the other hand, the technician punctures the skin and slowly injects the urine into the dorsal lymph sac, an area just below the juncture of the pelvic bones. After the injection, the frog is replaced in the container, which is labeled with the name of the patient and the time of the injection.

Although urine with a high titer of the chorionic gonadotropin will stimulate spermatogenesis in as little time as fifteen to twenty minutes, most workers believe that it is best to wait one hour before examining the frog urine, except in cases of extreme urgency. At the end of an hour, a drop of urine is removed from the cloacal orifice (bladder) of the frog by using a small pipette. Some of the workers studying this test used urine from the bottom of the jar, but we find it best to use the catheterized specimen because it is free from the debris which causes difficulty when examining for spermatozoa. This drop is placed on a clean slide, and examined for sperm under both the low and high power lens of a microscope. Finding one or more spermatozoa is evidence of a positive test. There are no degrees of positivity.

Frog spermatozoa are easily recognizable. They are relatively large—four times the size of human spermatozoa—have cigar-shaped bodies, and long tails. They are motile; even nonmotile spermatozoa, however, are indicative of a positive test.

Giltz and Miller caution that excessive dryness of the frog, or the use of large amounts of test fluid can cause spermatogenesis; this would indicate that these two conditions should be avoided in carrying out the tests. This is the first instance of discovery of spermatogenesis which occurs without the presence of chorionic hormone in the injected fluid (9).

If no spermatozoa are found, the frog is re-examined every hour until they are found, or until five hours have elapsed; then, if no spermatozoa are found, the test is considered negative. Early workers used two hours as the end point, but subsequent experimenters found positive reactions as late as five hours; so that time is now generally accepted as the end point.

Negative tests are evaluated in conjunction with the patient's symptoms. It is well to run simultaneous tests using two frogs. It is also advisable to repeat negative tests in four to seven days, because individual variations may cause a low titer of chorionic gonadotropin in the patient's urine at the time of the first test.

Siegler and Fein have presented a graph showing the chorionic gonadotropin hormone in the blood and urine of pregnant women. This graph indicates that the concentration begins about the 15th day, rises rapidly until the 30th day, and drops rapidly until the 90th day. It remains at a low level throughout the remainder of the pregnancy and drops to zero after delivery of the complete placenta (6).

Urine collected during the first trimester has been uniformly found to be positive, indicating that the results of the test correspond to the concentration of the hormone in the pregnancy urine. Since the hormone concentration declines after the third month, and many false-negative tests were found after this time, it has been concluded that it is useless to run the test thereafter. By the fourth month there are usually enough physical changes in the patient to make a positive diagnosis, without any need for biological testing.

### Evaluation of the Frog Test

This test has proved to be very useful and accurate in actual practice. Of the forty-one tests run by the writer, over a period of a year, there was only one false-negative test. There were no false-positive tests. The false-negative test occurred in a specimen from a patient whose menses had never been properly established (from four to six month intervals). On the basis of her continued nausea, the frog test was run. Although it was negative, a tentative diagnosis of pregnancy was made (as indicated by her general symptoms). The test was repeated a week later and was positive. On the evidence of previous studies, one may assume that the false-negative specimen had been taken when the titer of the chorionic gonadotropin was still very low.

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1 Dr. James Mashburn, personal communication. His information was based on experiments he conducted at the University of Arkansas Medical School in Little Rock, Arkansas.
On the basis of clinical evidence, all of the other tests have been proven accurate. The negative tests were run on patients with conflicting symptoms; some had tumors, others had periods of amenorrhea following emotional strain, and one had a possible ectopic pregnancy. It was not found necessary to use two frogs for each test, as recommended by some workers who conducted experiments.

In this series, it was not possible to tabulate the tests with reference to the number of days past the last menses because some of the tests were run for other physicians, and this information was not available. With one exception, however, they were all within the limits of the first trimester of pregnancy. In instances where the date of the last menses was known, the earliest positive reactions occurred in a patient whose menses were five days overdue. The other extreme occurred when the menses were three and one-half months overdue. The reaction in this case was barely positive and required five and one-half hours. Most of the positive reactions occurred within the first two-hour period, some before the third hour, and a few at four hours. A test, however, was not considered negative until it had been examined at the end of five hours.

Some workers report the use of blood serum for the injections, but it seems an unnecessary procedure; urine serves the purpose very well.

There are numerous medical reasons for needing to know definitely whether conception has taken place. One of the most important conditions in which it is necessary to have an early and accurate diagnosis is ectopic pregnancy. Delay in this case could mean the difference between life and death for the patient. In this condition, when a tubal mass is found and the frog test is positive, it is conclusive evidence of an ectopic pregnancy; surgery may be done prior to the rupture of the tube, preventing subsequent complications.

In threatened abortion, a positive pregnancy result with the frog test indicates that the placenta has not been completely detached from the uterine wall; therefore, conservative treatment is followed in an attempt to save the baby (11). If the abortion has been complete, the frog test becomes negative within ten to twelve hours, whereas the Friedman test remains positive for seven to ten days after the placenta is detached, and so is of little help in this case.

Tumors, cysts, infections, and other conditions often mask or imitate pregnancy. When the symptoms are conflicting, a reliable positive or negative pregnancy test is most helpful to the physician who is making his final diagnosis.

The *Rana pipiens* male frog test is simple to perform; it requires no special preparation of the urine to be tested; it is inexpensive; and there is no question of gradations of reaction. It is either positive or negative. This test is readily adaptable to the needs of the private physician, as well as to a large clinical practice until a newer test is found, this one meets all the criteria of adequacy.

REFERENCES


